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NEMATODES ASSOCIATED WITH DIPTERA IN SOUTH AUSTRALIA: A NEW SPECIES OF *FERGUSOBIA* CURRIE FROM A FERGUSONINID AND A NEW RECORD OF *SYRPHONEMA* LAUMOND & LYON FROM A SYRPHID

by KERRIE A. DAVIES* & JANINE LLOYD*

Summary

DAVIES, K. A. & LLOYD, J. (1996) Nematodes associated with Diptera in South Australia: a new species of *Fergusobia* Currie from a fergusoninid and a new record of *Syrphonema* Laumond & Lyon from a syrphid. *Trans. R. Soc. S. Aust.* **120**(1), 13-20, 31 May, 1996.

Fergusobia fisheri sp. nov., associated with a fly *Fergusonina* sp., is described from leaf galls on a hybrid of *Eucalyptus leucoxylon*. Like other species of *Fergusobia*, these nematodes are semi-obese and have a generation parasitic in the fly followed by a generation parasitic in the plant. The new species is distinguished from *F. tumifaciens* by its smaller size, larger manubrium on the spicule of the male, and smaller cephalic region and tail in the parthenogenetic female, with vulva and anus opening into cuticular depressions. *Syrphonema* sp., a nematode parasite of syrphid flies, is described and recorded for the first time outside France.

KEY WORDS: Taxonomy, Nematoda, *Fergusobia*, Diptera, *Syrphonema*, new species, new record.

Introduction

This paper describes a new species of the tylenchid nematode *Fergusobia*, the only genus of nematode known to parasitize both a plant and an insect (Maggenti 1981). It has a bipartite life cycle, with a generation parasitic in galls on Myrtaceae followed by one parasitizing the fergusoninid fly *Fergusonina* Malloch. Also reported is the first collection outside France of the rhabditid nematode genus, *Syrphonema*. Both nematodes were collected in Adelaide, South Australia.

Materials and Methods

Nematodes were collected from galls cut open in tap water, relaxed and fixed in hot FA 4:1. Insects were dissected in 0.85% saline, and nematodes from these were fixed as above. Nematodes were transferred from fixative to 1% glycerol in 30% ethanol in glass blocks and placed in a desiccator containing 96% ethanol for 2 days. For the following 14 days nematodes were kept at 40°C and the blocks were partially covered with glass lids. During the first three days, one or two drops of a solution of 5% glycerol in 95 ml ethanol were added four times daily. Slow evaporation was continued thereafter. For light microscopy, processed nematodes were mounted in glycerol on glass slides and examined using interference microscopy. Scanning electron microscopy studies were made. Nematodes were taken from glycerol, passed through a series of ethanol/glycerol solutions with increasing proportions of ethanol and washed three times with 100% ethanol. They were then dried using CO_2 in a critical point drier, mounted on stubs, sputter coated with approximately 30 nm of gold and viewed at 20 kV.

Measurements are in μm. Drawings and measurements were made from material mounted in glycerol using a camera lucida. Body width was measured at mid-length. Spicules were measured along the mid-line in lateral view. De Man's ratios, i.e. V = anterior end to vulva as percentage of body length, T = length of testis from cloaca to flexure as percentage of body length, a = length divided by greatest body width, b' = length divided by distance from anterior end to base of oesophageal glands, c = length divided by tail length, c' = tail length divided by width at anus were determined. Comparisons were made with described species using published descriptions, specimens of parthenogenetic females of *Fergusobia magna* Siddiqi (Queensland Museum (QM) G200512-200519) and specimens of all stages except parasitic females of *F. tumifaciens* Currie isolated by the authors from flower bud galls on *Eucalyptus camaldulensis* Dehn. at Urrbrae, South Australia (Waite Institute Nematode Collection (WINC) 943). The holotype of the new species is deposited in the South Australian Museum, Adelaide (SAM).

Taxonomic descriptions

Fergusobia fisheri sp. nov.
(FIGS 1-2)

Holotype: Parthenogenetic ♀, Black Forest, South Australia (34°57'S, 138°34'E), 3.viii.1993, W. Frost, collected from a leaf gall on a hybrid of *Eucalyptus leucoxylon* F. Muell., AHC207051 (SAM).

* Department of Crop Protection University of Adelaide, PMB 1 Glen Osmond S. Aust. 5064.

Paratypes: WINC 815, slides 1-28, including 20 parthenogenetic ♀♀, 25 preparasitic infective ♀♀, 39 parasitic ♀♀, 32 ♂♂, 59 juveniles, collected from leaf galls on a hybrid of *E. leucoxylon* at Black Forest between August and October 1993, by W. Frost.

Measurements: Table 1.

Description of parthenogenetic female (Figs 1A, H, 2A)

Occurs in leaf gall. Dorsally curved when relaxed by heat, with ventral side convex, to form open C-shape. Smaller than amphimictic preparasitic female. Cuticle weakly annulated, striated; lateral fields obscure. Cephalic region small relative to width of body at anterior end, off-set, lightly sclerotized; region appears roughly circular, unstriated, with 8 sectors; dorsal and ventral sectors less than half width of each of others; in side view sectors with rounded outline and no central elevation around stylet opening. Amphidial openings pore-like, situated near dorsal edge of lateral sector. Stylet slender; conus forming half length; small fusiform basal knobs. Orifice of dorsal oesophageal gland approximately 3 μm posterior to stylet knobs. Digestive tract with swollen anterior part with valve-like structure, followed by short narrow "isthmus" which widens abruptly to broader part associated with the oesophageal glands; oesophago-intestinal junction obscure. Lumen of tract broadens posterior to "valve" and again at level of secretory-excretory pore. Oesophageal glands large, occupying about three quarters of body width, extending over intestine to about 55% of total body length; dorsal gland with large nucleus. Secretory-excretory pore 50-92 μm from anterior end with short duct leading directly to excretory cell. Nerve ring at base of swollen anterior part of digestive tract. Hemizonid not seen. Reproductive tract with single gonad, prodelphic, extending to nerve ring; oviduct flexed in some females; uterus with quadricolumellar, often contains single egg, curves to join vagina, which is directed slightly forwards. Vulva usually conspicuous depressed transverse slit, but in some specimens, vulval lips protrude slightly. Cuticle wrinkled on ventral side just anterior to vulva in half specimens examined. Rectum simple tube, without obvious musculature; anus inconspicuous pore, usually associated with distinct indentation in the cuticle. Tail conoid, narrowing sharply to rounded tip, 1.0-1.4 times as long as anal body width. Phasmids not seen.

TABLE 1. *Measurements of* Fergusobia fisheri *sp. nov.*
Measurements in μm; n for each measurement in brackets below mean.

	Holotype	Paratypes											
	(parthenogenetic female)	Parthenogenetic females			Males			Pre-parasitic females			Parasitic females		
		mean	S.D.	range	mean	S.D.	range	mean	S.D.	range	mean	S.D.	range
Length	250	253 (12)	23.2	228-305	336 (16)	39.5	292-453	349 (25)	35.8	241-395	786 (20)	57.8	690-905
Width	50	37 (12)	2.1	33-40	37 (16)	3.7	32-46	38 (25)	6.2	28-49	112 (20)	7.9	105-128
Stylet length	10	9.4 (8)	0.38	9-10	9 (13)	1.0	8-11	8 (11)	1.4	6-11	nil	-	-
Anterior end to sec.-ex. pore	80	75 (9)	15.4	50-92	76 (6)	12.2	61-93	75 (5)	10.6	66-91	-	-	-
Tail length	15	14 (12)		13-16	31 (16)		23-42	30 (25)		21-49	nil	-	-
V	87	85 (12)	1.4	83-88	-	-	-	80 (25)	3.0	76-88	83 (18)	2.7	78-89
T	-	-	-	-	75 (16)	7.2	59-84	-	-	-	-	-	-
Spicule length	-	-	-	-	18 (17)	1.8	16-20	-	-	-	-	-	-
a	5	6.8 (12)	0.7	6.0-8.5	9.2 (16)	1.4	6.3-11.3	9.5 (25)	1.7	6.8-12.5	7.0 (20)	0.6	5.9-8.1
b'	1.8	1.9 (12)	0.15	1.8-2.1	2.8 (10)	0.41	2.3-3.4	3.2 (9)	0.42	2.8-4.0	-	-	-
c	15.6	15.1 (12)	4.4	10-25	12.9 (16)	1.5	11-17	12.9 (25)	4.4	7-23	-	-	-
c'	1.2	1.2 (12)	0.13	0.9-1.3	-	-	-	-	-	-	-	-	-

Description of infective pre-parasitic female (Fig. 1B, F, G)

Occurs in leaf gall, infects mature larval stage of fly. Anterior part of nematode straight when relaxed by heat; posterior part curved dorsally. Maximum body width at mid-length. Cuticle with inconspicuous annulations; strongly striated; lateral fields arising about one body width behind head, with irregular, broken striae, obscure when viewed with light microscope. Cephalic region continuous with body, weakly sclerotized. Stylet slender, weakly sclerotized with smaller basal knobs than in parthenogenetic female. Amphids not seen. Orifice of dorsal oesophageal gland approximately 3 μm posterior to stylet knobs. Secretory-excretory pore approximately half the distance along the oesophageal glands (65-90 μm from anterior end). Nerve ring at base of swollen anterior part of digestive tract. Hemizonid not seen. Oesophageal glands often obscure, elongate, occupying about half body width, extend over intestine to about 30% total body length. Anterior part of digestive tract swollen,

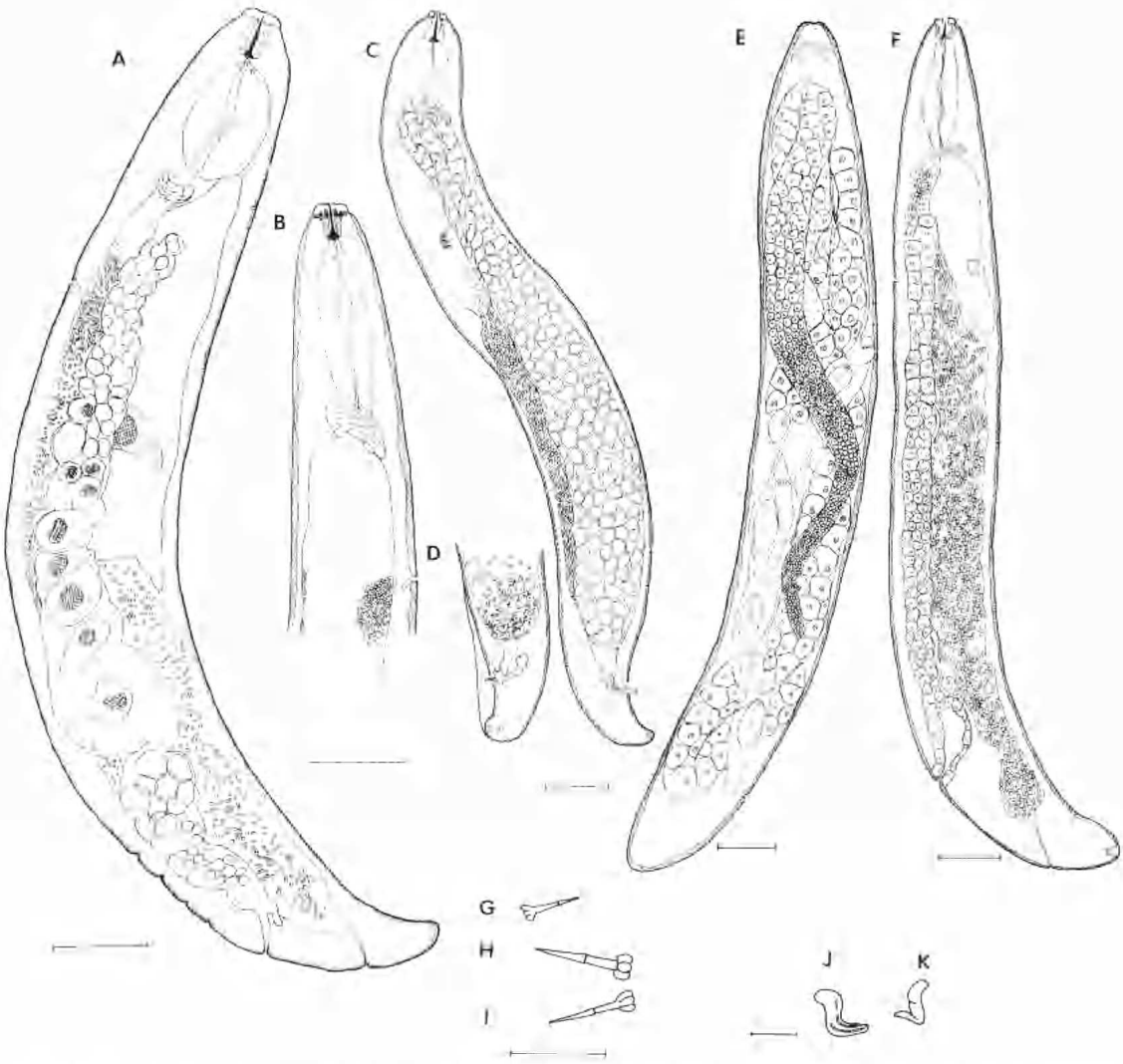
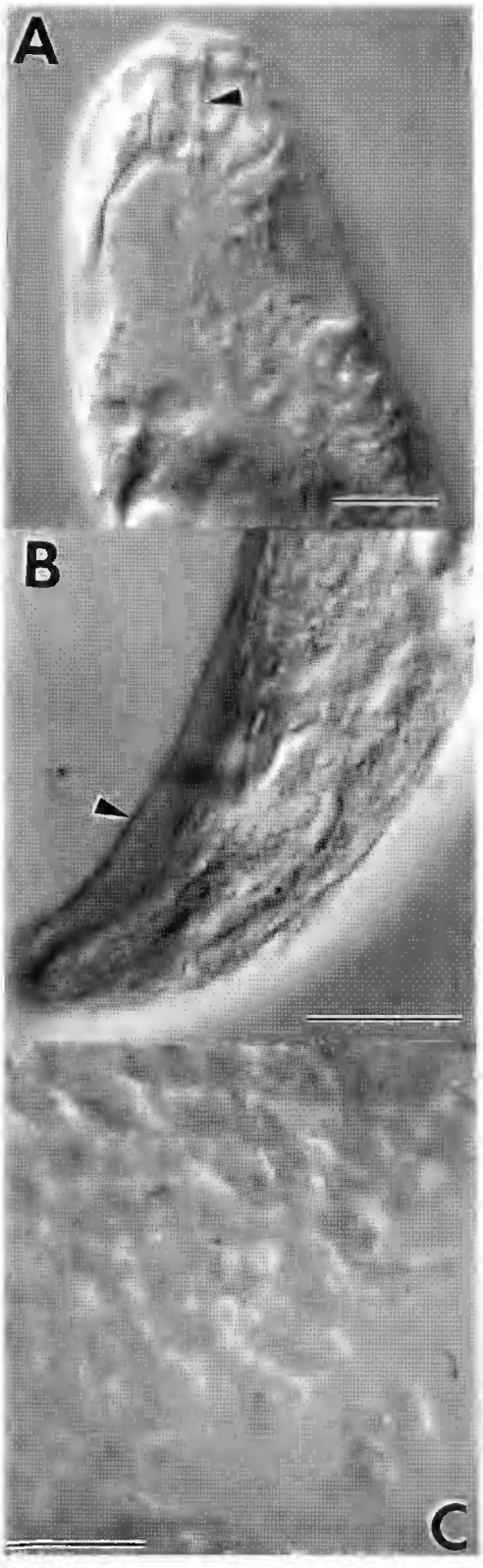


Fig. 1. *Fergusobia fisheri* sp. nov. A. Entire parthenogentic female. B. Head of pre-parasitic female. C. Entire male (bursa not seen due to angle at which tail viewed). D. Detail of male tail, ventro-lateral view, showing bursa and angulated spicule. E. Entire parasitic female. F. Entire pre-parasitic female. G, H, I. Stylets of pre-parasitic female, parthenogenetic female and male, respectively. J, K. Lateral and dorsal view, respectively, of spicule. Scale bars = 10 μm G H I J K, 20 μm A B C D F, 80 μm E.



non-muscular with valve-like structure. Intestine may contain many dense granules or lumen may be broad and empty, possibly reflecting nutritional status. Cells of intestinal walls have prominent nuclei with large nucleoli. Uterus extends almost to base of oesophageal glands, acting as spermatheca and packed with sperm; no post-vulval sac; vagina directed anteriorly, plugged with refractile material; oviduct short, often with flexure; ovary extending to nerve ring. Vulva transverse slit, inconspicuous; lips may be raised. Anus pore-like; rectum very small, non-muscular. Tail almost hemispherical. Numerous large nuclei scattered along length of nematode in epidermis.

Description of parasitic female (Fig. 1E)

Occurs in haemocoel in abdomen of fly. Epidermis thickened. Cephalic region may or may not be offset. Body swollen, sausage-shaped, obese, filled with hypertrophied reproductive organs. No stylet seen. Oesophagus and intestine degenerate, anus not seen. Ovary convoluted. Several eggs in uterus at one time. Vulva depressed transverse slit.

Description of male (Figs 1C, D, I, J, K; 2B, C)

Occurs in leaf gall. Body shape variable when relaxed by heat, posterior portion of body may be arched dorsally, tail curved ventrally. Cuticle with longitudinal striations, without annulations; lateral fields indistinct under light microscope, appear to be 3 or 4 incisures or several irregular striae. Cephalic region off-set, with lightly sclerotized framework. Stylet, oesophagus, intestine and secretory-excretory pore all as for parthenogenetic female. Oesophageal glands extend over intestine to about 35% of total body length. Reproductive tract with single testis, extending to nerve ring; extensive vas deferens, with amoeboid sperm. Bursa membranous, smooth, often difficult to see; extends to tail tip, appears to be peloderan; collapsed in specimens prepared for SEM, seen as wrinkled membrane lying on cuticle of tail region; variable length, usually arises 1.5-2 tail lengths anterior to cloaca, but in one specimen arose in anterior half of nematode. No genital papillae seen. Spicules robust, paired, angular near their middle so that manubrium and shaft appear to be perpendicular to each other in ventral view; manubrium large. No gubernaculum. Tail bluntly rounded.

Fig. 2. *Fergusobia fisheri* sp. nov. A. Parthenogenetic female head to show buccal region and stylet (arrow). B. Male tail showing bursa (arrow). C. Amoeboid spermatozoa from squashed male. Scale bars = 10 μm.

Diagnosis and relationships

F. fisheri sp. nov. is characterized by having a parthenogenetic female with the cephalic region small relative to the body width, with a flat terminal profile, the vulval slit in a conspicuous depression of the cuticle, the anal opening in a similar but smaller depression and a short tail (0.9-1.3 times anal body width) with a narrow cone shape. The male has angular spicules with a large manubrium.

F. fisheri sp. nov. differs from *F. jambophila* Siddiqi in having a flat cephalic region without a central conical elevation and angular spicules and from *F. indica* (Jairajpuri) and *F. magna* because the parthenogenetic female has a short tail. The anus opens into an obvious cuticular depression in the parthenogenetic female of *F. fisheri* sp. nov. but not in the other described species. *F. fisheri* sp. nov. assumes a similar shape to *F. tumifaciens* when heat-killed but is smaller (average length of parthenogenetic females 253 and 318 μm respectively and of males 336 and 420 μm. Measurements for *F. curriei* (=*tumifaciens*) from Fisher and Nickle 1968). The parthenogenetic female of *F. fisheri* sp. nov. has a smaller cephalic region relative to body width than *F. tumifaciens* and may have wrinkled cuticle on the ventral side of the body anterior to the vulva, absent in *F. tumifaciens*. The vulval slit is situated in a distinct cuticular depression in *F. fisheri* sp. nov. as in *F. jambophila* and *F. indica*, but not in *F. tumifaciens* or in *F. magna*. The volume of the tail of the parthenogenetic female is smaller in *F. fisheri* sp. nov. than in *F. tumifaciens*, having a narrower cone shape. The point of origin of the caudal alae is variable in *F. fisheri* sp. nov. males, in contrast to *F. tumifaciens*.

Etymology

Named after Dr J. M. Fisher, formerly of the Department of Crop Protection University of Adelaide.

Biology, life cycle and general comments

The nematode *F. fisheri* sp. nov. was found on leaf galls of the southern blue gum, *E. leucoxylon*, in association with an unknown species of the fergusoninid fly *Fergusonina* sp. Galls were first collected in early August 1993, followed by successive collections until October 1993 when no nematodes or flies were found. In the early August collection, galls contained many nematodes (juveniles, parthenogenetic and infective females and males) and fly larvae. Of 32 larvae dissected, the abdomen of two contained a total of three fertilized infective females and one male nematode. A week later, 14 fly larvae and 15 puparia were collected from galls and dissected. Six of the larvae contained two to four fertilized infective female nematodes. No nematodes were found in puparia. In early September, galls contained a few adult male and infective female nematodes. Puparia contained pharate adult flies. Nineteen pharate adult flies were dissected, eight of which were male and had no nematodes. However, 10 of the 11 female flies contained from 1-11 (average 6.4) mature parasitic female nematodes per fly. In six of these, eggs had been laid in the haemolymph and in most cases, the eggs were in the early stages of embryonic development. One fly, however, contained eggs in which the juvenile nematodes were well-formed.

These observations on the life cycle generally agree with those reported by Fisher & Nickle (1968) for *F. curriei* (=*tumifaciens*). No nematodes have been found in male flies. Eggs are laid in the haemolymph of the abdomen of the fly but it is not known how the fly deposits both insect eggs and nematode juveniles into *Eucalyptus* tissue. Presumably, these nematode juveniles develop into parthenogenetic females which lay eggs. It is possible that juveniles developing from the early eggs become males, as in *F. tumifaciens*. While the first collection of *F. fisheri* sp. nov. from leaf galls yielded all plant parasitic stages of the nematode, the fourth stage juveniles found were all female (distinguished from males by a more bluntly rounded tail and development of the uterus), suggesting that male development had occurred earlier. Fisher & Nickle (1968) stated that only fertilized infective-stage females of *F. curriei* (=*tumifaciens*) penetrated fly larvae but female and male *F. fisheri* sp. nov. have been found in fly larvae. *F. tumifaciens* may deposit eggs in the haemolymph of the fly before it emerges as an adult female (Currie 1937) or this may be delayed until after emergence of the fly from the gall (Fisher & Nickle 1968). Here, *F. fisheri* sp. nov. had produced eggs before the parasitised fly had emerged from the puparium.

Currie (1937) described one species, *F. tumifaciens*, associated with *Fn. carteri* Tonn., from leaf galls on *E. Stuartiana* (sic) F. v. M. He also observed minor morphological differences between nematodes collected from leaf bud, axil bud, stem tip and flower bud galls associated with a number of other fly species on more than a dozen species of eucalypt. He suggested "further work will show that the nematodes associated with the different flies have differences in structure which entitle them to be considered as different species" (Currie 1937). These differences included variation in the point of origin of the caudal alae and the size and position of the oesophageal glands. Observations of material held in the WINC confirm that these characters will be important in species differentiation. Fisher & Nickle (1968) redescribed *F. curriei* (=*tumifaciens*) from flower bud galls on *E. camaldulensis* Dehnh. associated with *Fn. tillyardi* Tonn. Of the other described species of *Fergusonina*, Siddiqi (1986) described *F. magna* from *Eucalyptus* stem galls associated with an unknown fly host and from soil under host trees, *F. indica* from soil in India

and *F. jambophila* from flower bud galls on *Syzygium cumini* (L.) Skeels associated with *Fn. syzygii* Harris.

The Waite Institute Nematode Collection contains specimens of *Fergusobia* collected from stem, bud and leaf galls on *Eucalyptus* from Adelaide and Mt Gambier, respectively, which appear to be undescribed species. Fisher (pers. comm.) found an undescribed species of *Fergusobia* in leaf galls on *E. camaldulensis* associated with *Fn. lockharti* Tonn. It is apparent, given the different forms of galls, that each of these undescribed species of nematode is associated with a different species of *Fergusonina*. Each, however, is not necessarily restricted to one species of *Eucalyptus*. Presumably the nematode has evolved mechanisms, which are probably host-specific, to escape the fly's immunological system during the generation in which it is an insect parasite. Thus, *Fergusobia*, like the genus *Anguina* Scopoli (Krall 1991) may have a high degree of host specificity, in this case, for the insect host. If so, Currie (1937) and Fisher & Nickle (1968) may not have described the same species of nematode, given that they were associated with different species of *Fergusonina*. Indeed, Currie refers to the margins of the caudal alae of males of his specimens of *F. tumifaciens* as being slightly crenate, a character not observed by Fisher & Nickle (1968) nor in specimens isolated from *E. camaldulensis* by the present authors. Also, the oesophageal glands of the parthenogenetic female of the specimens from *E. camaldulensis* are longer than in Currie's description and drawing of *F. tumifaciens*.

The structure of the digestive tract of *Fergusobia* remains unclear. Given the small size of these nematodes, and their typical dark colouration, it is very difficult to distinguish the anterior parts of the tract. Fisher & Nickle (1968) described the anterior part of the oesophagus as swollen, narrowing abruptly to form a short isthmus at the level of the nerve ring, then broadening again to contain the large glands. They believed that the oesophago-intestinal junction occurred at about the level of the secretory-excretory pore. Siddiqi (1986) interpreted the anterior swelling of the digestive tract as a "pseudo-pharynx" and believed that the valve-like structure it contains marked the junction of the oesophagus and intestine. An electron microscope study of the anterior part of the digestive tract of *Fergusobia* is needed to decide which of these interpretations is correct.

Syrphonema sp.
(FIG. 3)

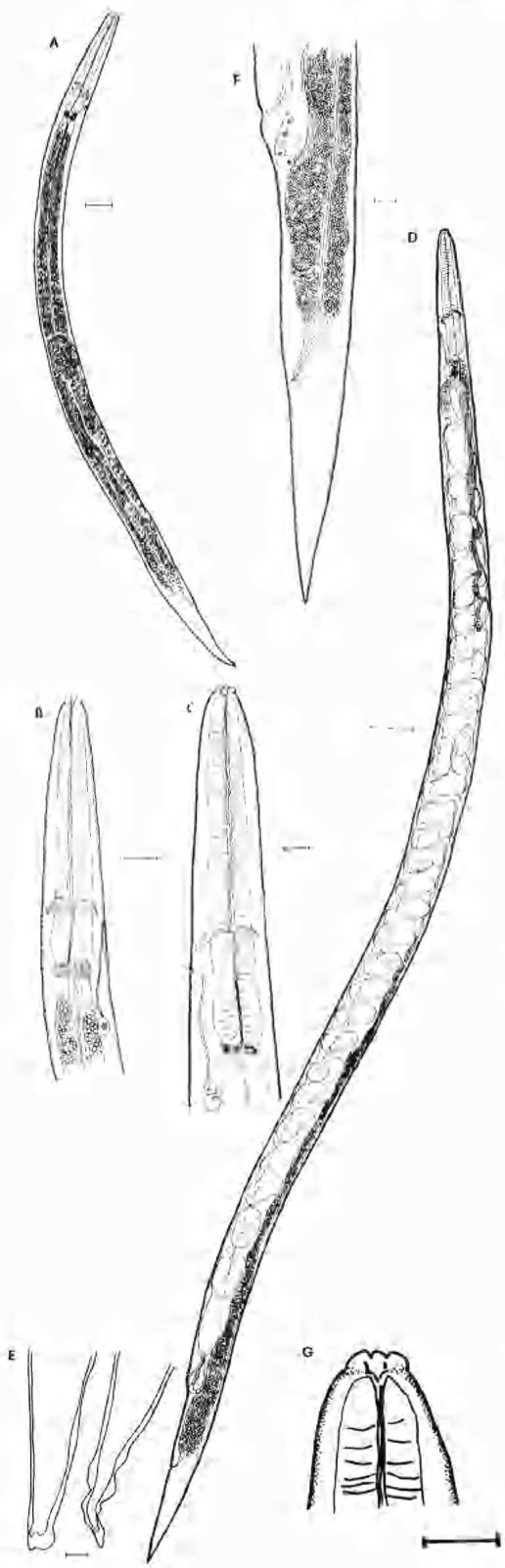
Measurements: Table 2.

Description of female (Fig. 3C, D, E, F, G)

Nematodes straight or slightly C-shaped when relaxed by gentle heat. Cuticle has longitudinal grooves with many fine transverse markings; often appears loose at head and/or tail; lateral lines large (31 μm wide, 27-33 μm), with smooth ribbon-like appearance and single central ridge. Lip region often indistinct; six separate lips, each with small papilla. Stoma cup-shaped; cheilostom reduced; promesostom about 4 μm long, and about same width; thickening of cuticle on ventral side of metastom, which makes stoma asymmetrical, and may form a rhabdion; telostom present. Amphid openings at base of lateral lips. Oesophagus rectilinear; no true bulb; vestigial valve present. Nerve ring situated in posterior third of oesophagus. Secretory-excretory pore opening just behind nerve ring; prominent excretory cell just behind oesophago-intestinal junction. Deirids not seen. Hemizonid just posterior to nerve ring. Intestinal lumen lined with refractive material from oesophago-intestinal junction to rectal valve; lumen wide in young females but narrower in older. Three rectal glands. Reproductive tract with single gonad, amphidelphic, with genital tube running anteriorly; ovoviviparous; long uterus, extending almost to point of flexure of genital tube just behind oesophago-intestinal junction; oviduct extending back down dorsal side of body; small post-uterine sac present; no true spermatheca, sperm not seen; vulva with transverse opening, well-developed associated musculature; posterior vulval lip often more prominent than anterior; vagina not directed anteriorly or posteriorly. Phasmids not seen. Tail conical, terminus variable (Fig. 3).

TABLE 2. *Measurements for adult females of* Syrphonema *sp. isolated from Adelaide.*
All measurements in μm

	Length	Width	Anterior end to base of bulb	Tail length	Anterior end to vulva	V	a	b	c
mean	1470	49.7	140.5	106.0	1200	81.6	29.8	10.6	13.8
n	12	12	10	10	12	12	12	10	10
S.D.	236	6.8	20.5	16.9	220	5.7	3.6	1.7	1.5
range	1230-1890	32-56	108-171	83-130	910-1620	66.9-86.5	25-36	8.8-13.4	11.4-16.0



Description of juveniles (Fig. 3A, B)

Second stage juveniles 573 μm (473-624; n=8); third stage juveniles 830 μm (806-873; n=3); fourth stage juveniles 1062 μm (920-1260; n=7).

As for adult females, except that the lateral lines consist of a single central ridge only. Gonad primordium well-developed in third and fourth stage juveniles, developing uterus particularly obvious in fourth stage nematodes, enabling rapid determination of the various juvenile stages.

Collector, host and locality

The nematodes were dissected from the intestine of two females of the syrphid fly *Simosyrphus grandicorinis* (Macquart), collected on sow thistle, *Sonchus* L. sp., at the Waite Campus of the University of Adelaide, Glen Osmond in January and December 1993 by Mr E. Soleyman. Nematode specimens are held in the WINC 687.

Biology and general comments

A search of Helminthological Abstracts suggests that this is only the second record of the genus *Syrphonema*, erected by Laumond & Lyon (1971), and the first outside France. Its occurrence in South Australia suggests that the genus may have a cosmopolitan distribution. Laumond & Lyon (1971) collected *S. intestinalis* from the digestive tracts of 12 species of syrphid flies. The infected flies found here were part of collections made in a study of the biology of the syrphid flies, *S. grandicorinis* and *Melangyna viridiceps* (Macquart). No nematodes were seen in dissections of 305 *M. viridiceps* and only two of 105 *S. grandicorinis* dissected contained nematodes (Soleyman pers. comm.) suggesting that the infection rate is naturally low. It is not known what effect, if any, infection has on the survival and reproductive capacity of the fly.

The nematodes described here from South Australia were classified as *Syrphonema* on the basis of the host fly, rectilinear oesophagus without a bulb and with a vestigial valve and because the female is ovoviviparous and has a posterior vulva. In the absence of males, it is not possible to decide if the nematode is *S. intestinalis* or a new species. The body lengths of the South Australian and French forms suggest that the former were smaller, but the De Man ratios are very

Fig. 3. *Syrphonema* sp. A. Entire fourth stage juvenile. B. Anterior of fourth stage juvenile. C. Anterior of adult female. D. Entire adult female. E. Variable tail shapes of adult females. F. Vulva and tail of female. G. Stoma of adult female. Scale bars = 10 μm B C E F G, 20 μm A, 50 μm D.

close (Table 2). Some apparent morphological differences have been observed. Laumond & Lyon described the stoma of their specimens as reduced and "vestibule-formed"; in the nematodes described here the stoma was cup-shaped but asymmetric with cuticular thickening (possibly a rhabdion) of the ventral metastom. The nerve ring seems to be located more posteriorly in the South Australian than in the French specimens. Again, the drawing of the female in Laumond & Lyon (1971) does not show a post-uterine sac or a protuberant posterior vulval lip, both present in the specimens examined here. While Laumond & Lyon state that *S. intestinalis* does not have a spermatheca, they have drawn a structure, also seen in South Australian females, which could function as a spermatheca. This is an apparent modification of the reproductive tube, just on the uterine side of the flexure of the oviduct; however, no sperm were seen. An attempt to obtain material from France for comparative studies was unsuccessful.

Acknowledgments

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